PRODUCT INFORMATION & MANUAL

Glycogen Assay Kit (Colorimetric)

NBP3-24501

For research use only.
Not for diagnostic or therapeutic procedures.

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Glycogen Assay Kit
(Colorimetric)

Catalog No: NBP3-24501

Method: Colorimetric method

Specification: 100 Assays (Can detect 96 sampleswithout duplication)

Instrument: Spectrophotometer

Sensitivity: 1.80 mg/g liver tissue, 0.36 mg/g muscle tissue

Detection range: 1.80-180 mg/g liver tissue, 0.36-36 mg/g muscle tissue

Average intra-assay CV (%): 3.7

Average inter-assay CV (%): 7.3

Average recovery rate (%): 101

- ▲ This kit is for research use only.
- ▲ Instructions should be followed strictly, changes of operation may result in unreliable results.
- ▲ Please kindly provide us the lot number (on the outside of the box) of the kit for more efficient service.

General information

▲Intended use

This kit can be used to measure the glycogen content in animal liver and muscle samples.

▲Background

Glycogen is mainly produced by liver and muscle. Glycogen content is mainly regulated by glycogen synthase and glycogen phosphorylase. In the liver, glycogen acts as a glucose store for other tissues and maintains blood glucose levels. In muscle, glycogen is mainly used as energy for the supply of adenosine triphosphate (ATP) during muscle contraction. Due to the lack of glucose-6-phosphatase, muscle glycogen cannot maintain the level blood glucose.

▲Detection principle

Under the presence of concentrated sulfuric acid, glycogen can be dehydrated to furfural derivatives. Furfural derivatives can form blue compound with anthracenone. The concentration of the compound can be measured by colorimetric quantification at 620 nm with glucose standard buffer of same treatment. Glycogen is quite stable in concentrated alkali solution. Heating the tissue sample in concentrated alkali solution before color development will remove other components and keep the glycogen.

▲Kit components & storage

Item	Component	Specification	Storage
Reagent 1	Alkali Reagent	50 mL × 2 vials	2-8℃ , 12 months
Reagent 2	Glucose Standard Solution	1 mL × 1 vial	2-8℃ , 12 months
Reagent 3	Anthracenone	Powder × 12 vials	2-8℃ , 12 months, shading light
Note: The reagents must be stored strictly according to the preservation conditions in the above table. The reagents in different kits cannot be mixed with each other.			

▲Materials prepared by users

Instruments

Spectrophotometer (620 nm), Micropipettor, Incubator, Vortex mixer, Centrifuge

Reagents:

Double distilled water, Normal saline (0.9% NaCl), Concentrated sulfuric acid

⚠ Safety data

Some of the reagents in the kit contain dangerous substances. It should be avoided to touch the skin and clothing. Wash immediately with plenty of water if touching it carelessly. All the samples and waste material should be treated according to the relevant rules of laboratory's biosafety.

⚠ Precautions

Before the experiment, please read the instructions carefully, and wear gloves and work clothes.

▲ The key points of the assay

1. This experiment must be done in glass tubes.
2. The temperature of the water bath should be above 95°C.

Pre-assay preparation

▲ Reagent preparation

1. Preparation of 0.01 mg/mL glucose standard solution:
   Dilute the reagent 2 with double distilled water for 100 times. Prepare the fresh solution before use. It can be store at 2-8 ℃ for a day.

2. Preparation of reagent 3 working solution:
   Dissolve a vial of reagent 3 with 20 mL concentrated sulfuric acid (self-prepared) and mix fully. Prepare the fresh solution before use. It can be store at 2-8 ℃ with shading light for 2h. If the reagents appear darkened color, it should be abandon.

[Notes]:

(1)The concentrated sulfuric acid must be 95%-98% analytical reagent opened recently (the concentration will decrease if it has been opened for a long time).

(2)The container and graduated cylinder must be absolute dry. Otherwise reagent 3 cannot be dissolved thoroughly.

(3)Add reagent 3 powder into the beaker, then add about 10 mL concentrated sulfuric acid. Press with a glass rod to pulverize the powder and improve dissolving. Add the remaining concentrated sulfuric acid and mix thoroughly.

(4)Pay attention to personal safety protection.

▲ Sample preparation

1. Sampling: Wash the fresh liver or muscle tissue sample with saline and dry with filter paper. Weigh the sample. It is recommended that the weight of the sample should be less than 100 mg.
2. Hydrolysis: Add the reagent 1 into a tube with a ratio of Weight (mg): Volume (μL) =1:3. Heat the tube in boiling water bath for 20 min. Cool the tube with running water.

   Examples: according to the ratio of w: v=1:3, 75 mg of liver tissue sample should be mixed with 225 μL of reagent 1. 85 mg of liver tissue sample should be mixed with 255 μL of reagent 1.

   *Notes: Seal the tube with preservative film to avoid water evaporation. Make a small hole on the film to allow vapour expanding and contracting.
3. Prepare the hydrolyzed glycogen testing solution for measurement

   Concentration of the liver glycogen testing solution is 1%, the volume of the double distilled water added should be: liver weight × 100 - liver weight×4* = liver weight × 96.

   Concentration of the muscle glycogen testing solution is 5%, the volume of the double distilled water added should be: muscle weight × 20 - muscle weight × 4*= muscle weight × 16.

   Notes: 4* is the volume of the dehydrated sample and Reagent 1 mixture. For example: weigh 80 mg liver tissue, add 240 μL reagent 1 to hydrolyze, it should add 7680 μL double distilled water to prepare 1% liver glycogen testing solution.

▲Dilution of sample

It is recommended to take 2~3 samples with expected large difference to do pre-experiment before formal experiment and dilute the sample according to the result of the pre-experiment and the detection range (1.80-180 mg/g liver tissue, 0.36-36 mg/g muscle tissue). The diluent could be double distilled water or normal saline.

Assay protocol

▲Detailed operation steps

(1) Blank tube: Take 1 mL of double distilled water into a 10 mL glass tube

Standard tube: Take 1 mL of 0.01 mg/mL glucose standard solution into a 10 mL glass tube.

Sample tube: Take 0.1 mL of liver/ muscle hydrolyzed glycogen testing solution in sample preparation step into a 10 mL glass tube and add 0.9 mL of double distilled water.

Note: this experiment must be carried out with glass tubes.

(2) Add 2.0 mL of reagent 3 working solution and oscillate fully with vortex mixer (The reagent 3 should be added slowly after addition of standard or sample).

(3) Fasten the tubes with plastic film and make a small hole, and incubate the tubes in 100℃ water bath for 5 min, then cool the tubes with running water immediately.

Notes: the mixture must be mix fully after adding the reagent 3 working solution, then heat the tubes in boiling water bath. Otherwise floccule will be formed during heating.

(4) Set the spectrophotometer to zero with double distilled water and measure the OD value of each tube at 620 nm with 1 cm optical path cuvette.

▲Summary operation table

	Blank tube	Standard tube	Sample tube
Double distilled water (mL)	1.0		0.9
0.01 mg/mL Glucose standard solution (mL)		1.0	
Liver/ muscle hydrolyzed glycogen testing solution (mL)			0.1
Reagent 3 working solution (mL)	2.0	2.0	2.0
Oscillate fully, fasten the tubes and make a small hole, and incubate the tubes in 100℃ water bath for 5 min, then cool the tubes to room temperature. Set the spectrophotometer to zero and measure the OD value of each tube.			

▲Calculation

$$\text{Glycogen content (mg/g tissue)} = \frac{\Delta A_1}{\Delta A_2} \times m \times f \times 10^{*} \div 1.11^{*}$$

Note:

ΔA_1: $OD_{Sample} - OD_{Blank}$

ΔA_2: $OD_{Standard} - OD_{Blank}$

m: The content of standard (0.01 mg)

f: Dilution factor of sample in sample preparation. For example, 1% liver glycogen testing solution, f = 100; 5% muscle glycogen testing solution, f = 20

10*: The dilution factor of reaction system

1.11*: The coefficient for converting the glycose concentration to glycogen concentration. The color degree of 100 μg of glycogen developed with anthracenone is equal to 111 μg of glycose with same treatment.

Appendix I Data

▲Example analysis

For mouse liver tissue, carry the assay according to the operation table.

The results are as follows:

The average OD value of the sample is 0.358, the average OD value of the blank is 0.181, the average OD value of the standard is 0.331, and the calculation result is:

Glycogen content(mg/g tissue) = (0.358-0.181)÷(0.331-0.181)×0.01×100×10÷1.11
=10.63 (mg/g tissue)

Appendix II References

1. Akram M, Asif H M, Akhtar N, et al. Glycogen metabolism and glycogen storage diseases A review. Journal of Medicinal Plants Research, 2011, 5(20): 4980-4983.
2. Bollen M, Keppens S, Stalmans W. Specific features of glycogen metabolism in the liver. Biochemical Journal, 1998, 336(Pt 1) (1): 19-31.
3. Roden M, Perseghin G, Petersen K F, et al. The roles of insulin and glucagon in the regulation of hepatic glycogen synthesis and turnover in humans. Journal of Clinical Investigation, 1996, 97(3): 642-648.
4. Thorburn A, Andrikopoulos S, Proietto J. Defects in liver and muscle glycogen metabolism in neonatal and adult New Zealand obese mice. Metabolism Clinical & Experimental, 1995, 44(10): 1298-1302.